Published in final edited form as:

Exp Toxicol Pathol. 2014 October ; 66(8): 377–382. doi:10.1016/j.etp.2014.05.005.

PCB 126 PERTURBS HYPOXIA-INDUCED HIF-1 α ACTIVITY AND GLUCOSE CONSUMPTION IN HUMAN HEPG2 CELLS

Sabine U. Vorrink^{a,b}, Ehab H. Sarsour^b, Alicia K. Olivier^c, Larry W. Robertson^{a,d}, Prabhat C. Goswami^{a,b}, and Frederick E. Domann^{a,b,c,*}

^aInterdisciplinary Graduate Program in Human Toxicology, The University of Iowa, Iowa City, Iowa, USA

^bDepartment of Radiation Oncology, The University of Iowa, Iowa City, Iowa, USA

^cDepartment of Pathology, The University of Iowa, Iowa City, Iowa, USA

^dDepartment of Occupational and Environmental Health, The University of Iowa, Iowa City, Iowa, USA

Abstract

Aerobic organisms strongly depend on the availability of oxygen for respiration and countless other metabolic processes to maintain cellular homeostasis. Under certain conditions, the amount of available oxygen can be limited. To support survival in environments with limited oxygen supply, hypoxia-inducible factors (HIFs) reprogram vital components of cellular metabolism. HIF-1 α is an important mediator of acute and adaptive responses to hypoxic stress. Interestingly, the heterodimeric partner required by HIF-1 α to function as transcription factor, known as ARNT, is also an essential part of the aryl hydrocarbon receptor (AhR) transcription factor complex. Thus, via ARNT a crosstalk exists between these two pathways that might affect HIF-1 α -mediated processes. In this study we sought to assess the effect of the AhR agonist PCB 126 on HIF-1 α activity as well as on HIF-1 α -regulated targets involved in cellular metabolism in human HepG2 cells. Our results show that PCB 126 reduced HIF-1 α localization to the nucleus. Furthermore, in an *in vivo* setting, rats exposed to parenteral PCB 126 also displayed reduced hepatocyte nuclear localization of HIF-1 α . Additionally, HepG2 cells exposed to PCB 126 displayed reduced hypoxia-regulated HRE-luciferase reporter gene expression as well as a reduction in glucose consumption in conditions of hypoxia. In summary, this study reveals that HIF-1 α -regulated cellular metabolic processes are negatively affected by PCB 126 which might ultimately affect adaptive responses and cell survival in hypoxic environments.

© 2014 Elsevier GmbH. All rights reserved.

*Corresponding author: Frederick E. Domann, Ph.D., Department of Radiation Oncology, B180 Medical Laboratories, The University of Iowa, Iowa City, IA 52242, USA. Telephone: +1 319-335-8018. Fax: +1 319-335-8039. frederickdomann@uiowa.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest Statement

The authors declare that no conflicts of interest exist.

Keywords

cellular metabolism; hepatocytes; low oxygen environments; nuclear localization; polychlorinated biphenyls

Introduction

Molecular oxygen (O₂) is an essential component of cellular metabolism and homeostasis of aerobic organisms; it functions as a key substrate for oxidative phosphorylation, enzymatic activity and many other biochemical processes. Conditions of reduced oxygen availability, or hypoxia, can occur in various physiological and pathological states and it is crucial for living cells to adapt to these conditions in order to ensure survival. Important mediators of responses to hypoxic stress are the hypoxia-inducible factors (HIFs) which are regulated by oxygen availability. Of the three known isoforms (HIF-1 α , HIF-2 α , and HIF-3 α), HIF-1 α is ubiquitously expressed and induces a broad range of target genes (Majmundar *et al.*, 2010). In environments of sufficient oxygen supply (> 6% oxygen), HIF-1 α is hydroxylated by oxygen-dependent prolyl hydroxylase domain-containing enzymes (PHDs). Hydroxylation leads to subsequent ubiquitination by von Hippel-Lindau (VHL) proteins and ultimately proteasomal degradation (Ohh *et al.*, 2000; Kewley *et al.*, 2004; Dery *et al.*, 2005). However, if oxygen is scarce, the initial hydroxylation reaction of PHD enzymes is impaired, which allows HIF-1 α to accumulate. To function as a transcription factor, non-hydroxylated HIF-1 α translocates to the nucleus where it forms a heterodimer with the nuclear protein HIF-1 β , also known as the aryl hydrocarbon receptor nuclear translocator (ARNT) (Kewley *et al.*, 2004). The HIF-1 α :ARNT heterodimer induces target genes that contain a hypoxia response element (HRE) in their sequence (Mole *et al.*, 2009; Majmundar *et al.*, 2010). HIF-1 α regulated target genes are involved in various physiological processes including angiogenesis (vascular endothelial growth factor, VEGF), proliferation (erythropoietin, EPO), invasion and metastasis (E-cadherin, CDH1), or metabolism (glucose transporter 1 and 3, GLUT1/3; hexokinase 1 and 2, HK1/2; lactate dehydrogenase A, LDHA) (Semenza, 1999; Rankin and Giaccia, 2008). The induction of metabolic genes is an important mechanism for cell protection under stress. In order to ensure cell survival during limited oxygen availability, HIF-1 α reprograms important components of cellular metabolism (Majmundar *et al.*, 2010). Furthermore, carcinoma cells within tumors, which often have hypoxic regions, show changes in the expression of HIF-1 α -regulated glucose transporters and glycolytic enzymes (Gordan *et al.*, 2007; Majmundar *et al.*, 2010). For example, GLUT1 and GLUT3 are important targets of HIF-1 α and have been shown to be affected in cancer (Maxwell *et al.*, 1997; Macheda *et al.*, 2005; Wood *et al.*, 2011). Furthermore, cancer cells often show elevated levels of HIF-1 α and HIF-2 α as well (Rankin and Giaccia, 2008).

Interestingly, ARNT, the heterodimeric partner of HIF-1 α , is involved in other cellular signaling pathways as well, including the aryl hydrocarbon receptor (AhR) signaling pathway. In a recent study, our lab showed that AhR function and target gene expression upon exposure to the AhR ligand 3,3',4,4',5-pentachlorobiphenyl (PCB 126) were significantly inhibited by prior exposure to hypoxia. We further showed that conversely,

PCB 126 inhibited the expression of a HRE-regulated luciferase reporter gene under hypoxia (Vorrink *et al.*, 2013). Few studies have assessed the effects of PCB exposure on the hypoxia signaling pathway. A study by Kraemer and Schulte assessed the effect of PCB 77, another dioxin-like PCB, on hypoxia-induced glycolytic enzyme activity in fish and found that PCB treatment decreased enzyme activity (Kraemer and Schulte, 2004). However, it is not known if PCB 126 affects hypoxia-regulated responses of HIF-1 α . Based on these findings, we sought to assess whether PCB 126 has an effect on the hypoxia signaling pathway. This study was designed to assess the effect of PCB 126 on HIF-1 α as well as on HIF-1 α -regulated cellular processes, especially targets involved in cellular metabolism in liver hepatocytes, a major site of PCB metabolism. Oxygen tension plays an important role in the liver which under physiological conditions contains regions with varying oxygen concentration in anatomically defined zones (Oinonen and Lindros, 1998). Furthermore, the liver is a major site of cellular metabolic processes, including glucose and PCB metabolism. HIF-1 α is a crucial regulator of adaptive cellular processes to hypoxia in the liver, and plays an important role in cancer. Therefore, it is important to analyze whether and how exposure to environmental chemicals that act as AhR agonists might affect HIF-1 α and hypoxia signaling.

Materials and Methods

Chemicals and PCB treatment

3,3',4,4',5-pentachlorobiphenyl (PCB 126) was kindly provided by Dr. H. J. Lehmler of the Iowa Superfund Research Program at The University of Iowa. PCB 126 stock solutions were prepared in dimethyl sulfoxide (DMSO) and added to the cell culture medium at a final concentration of 3 μ M. All exposures were performed in serum-free medium. Control cultures were left untreated. 2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-Dglucose (2-NBDG) was purchased from Sigma (St. Louis, MO).

Cell lines and cell culture conditions

Human hepatocellular carcinoma (HepG2) cells were purchased from the American Type Culture Collection (ATCC) (Manassas, VA) and were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin. Cell culture in a normal oxygen environment was performed in a humidified 37°C incubator with 5% CO₂. For hypoxic cell culture, cells were incubated in a 37°C, humidified Bactron anaerobic/environmental chamber (SHEL LAB, Cornelius, OR) flooded with a 1% O₂, 94% N₂ and 5% CO₂ certified gas mixture. Cells were kept in hypoxia for 16 h prior to treatment with PCB 126 and were kept in hypoxia for the duration of the treatment. Hypoxic exposure was monitored by measuring HIF-1 α protein expression in cells grown in normoxia or hypoxia using an anti-HIF-1 α antibody (ab2185, Abcam, Cambridge, MA) at 1:500 dilution.

Animal treatment and HIF-1 α immunostaining

Paraffin-embedded livers from untreated and PCB 126 treated rats were kindly provided by Dr. Larry Robertson at The University of Iowa. Rats were originally treated as described (Lai *et al.*, 2012). Briefly, male Sprague-Dawley rats, fed a standard AIN-93G diet, received

a single intraperitoneal injection of either vehicle (stripped corn oil, 5 ml/kg body weight) or vehicle with 5 μ mol/kg body weight (1.63 mg/kg body weight) PCB 126. After two weeks rats were euthanized and tissues prepared as described (Lai *et al.*, 2012). Formalin-fixed, paraffin-embedded liver sections (4 μ m) were deparaffinized and rehydrated. Slides were retrieved in citrate buffer (pH 6.0) using a Decloaking Chamber (Biocare, Concord, CA). Endogenous peroxidase was quenched with 3% hydrogen peroxide for 8 min followed by incubation in 5% horse serum. Anti-HIF-1 α mouse monoclonal antibody (NB100-105, Novus Biologicals, Littleton, CO at a dilution of 1:50) was applied for 2 h in DAKO buffer at room temperature. Slides were rinsed and then incubated with DAKO Mouse Envision HRP System Reagent for 30 min. Slides were then developed with DAKO DAB plus for 5 min followed by DAB Enhancer for 3 min. A negative control slide was stained using the same procedure, omitting the primary antibody.

Isolation of cytoplasmic and nuclear extracts and Western Blot

HepG2 cells were left untreated or treated with PCB 126 prior to incubating cells in hypoxia for 24 h. Cells were washed with 1 $\times$ phosphate-buffered saline (PBS) and harvested with 1 $\times$ PBS containing 1 mM PMSF. After centrifugation, the pellet was resuspended in hypotonic lysis buffer (10 mM Tris HCl, pH 7.5, 2 mM MgCl₂, 3 mM CaCl₂, and 0.32 M sucrose) plus 10 mM NaF, 1 mM NaVO₄, 1 mM PMSF, and 1 $\times$ protease inhibitor cocktail (Roche; Complete Mini). Following a 15 min incubation of ice, cells were lysed by the addition of 0.87% NP-40. Cells were centrifuged and the supernatant was collected (cytoplasmic extract). After three washes with 1 $\times$ PBS containing 1 mM PMSF and 0.8% NP-40, the nuclear pellet was resuspended in 2 $\times$ packed cell volume equivalent of RIPA cell-lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 0.1% SDS, 0.5% Na Deoxycholate, 1% TX-100) containing 10 mM NaF, 1 mM NaVO₄, 1 mM PMSF, and 1 $\times$ protease inhibitor cocktail. The cells were sonicated in a cup sonicator and centrifuged. The supernatant was collected (nuclear extract) and the protein concentration was measured using a Bradford assay. Samples were stabilized with SDS sample buffer and boiled for 2 min prior to electrophoresis. Samples were separated on 7–12% SDS-polyacrylamide gels and were subsequently electrotransferred onto a nitrocellulose membrane (BioRad, Hercules, CA). The membrane was incubated overnight at 4°C with an anti-HIF-1 α antibody (ab2185, Abcam) at 1:500 dilution. To ensure equal protein loading, a human beta-tubulin antibody (Developmental Studies Hybridoma Bank, The University of Iowa, IA) and an anti-Histone H3 antibody (ab1791, Abcam) were used. Goat anti-rabbit IgG and goat anti-mouse IgG (Millipore, Billerica, MA) were used as secondary antibodies. Bands were visualized by chemiluminescence on a Typhoon FLA 7000 using ECL plus reagent (Pierce, Rockford, IL). For quantification, ImageJ image analysis software was used (Schneider *et al.*, 2012). Intensities of nuclear HIF-1 α were normalized to nuclear histone H3 and are depicted relative to untreated cells in hypoxia.

HRE-luciferase reporter assay

HepG2 cells at ~80% confluence were transfected with a HRE-luciferase reporter vector (Salnikow *et al.*, 1999) (4 μ g) and Renilla luciferase vector (4 μ g) using the Lipofectamine 2000 transfection reagent protocol (Invitrogen, Grand Island, NY). After 6 h, the medium was removed and replaced with fresh DMEM supplemented with 10% FBS. The next day,

cells were split and treated with PCB 126 for 4 h prior to transferring cells to hypoxia. After 10 h in hypoxia, cells were lysed and analyzed using the dual-luciferase reporter assay system (Promega). A Tecan SpectraFluor Plus luminometer was used to measure luminescence from whole cells lysates.

Glucose consumption

Glucose consumption was measured using the fluorescent glucose analog 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) (Sigma). HepG2 cells were incubated in serum-free low glucose DMEM (1 g/L) supplemented with penicillin-streptomycin. Cells were left untreated or were treated with PCB 126 for 4 h prior to transferring cells to hypoxia for 48 h. Untreated control cultures were left in normoxic conditions. After 48 h, cells were washed with low glucose DMEM supplemented with 10% FBS and fresh low glucose DMEM supplemented with 10% FBS and 20 μ M 2-NBDG was added to the cells. After 2 h, the medium was removed and the cells were detached from the plate with TrypLE Express and collected by centrifugation. Cells were washed twice with $1 \times$ PBS. After resuspension in 500 μ l $1 \times$ PBS, the cells were filtered through a 35 μ m mesh and analyzed by flow cytometry. The 2-NBDG fluorescence was recorded using a LSR Violet Flow Cytometer. 20,000 cells were analyzed per sample. Samples were normalized to control cells in normoxia to yield the relative glucose uptake values.

Statistical analysis

Experimental groups were analyzed using a t-test to determine significant differences between groups. Unless otherwise noted, three independent biological replicates ($n = 3$) were used for each group. A statistical probability of $p < 0.05$ was considered significant.

Results

PCB 126 affects the cellular localization of HIF-1 α in rat livers

Given the interaction between the AhR and hypoxia signaling pathways and the broad effects of PCB exposure, we analyzed whether PCB 126 could affect HIF-1 α , an important regulator of responses to hypoxia. To assess HIF-1 α expression and localization, liver sections of untreated and PCB 126 treated rats were stained for HIF-1 α (Figure 1). In control rat livers, we observed a faint diffuse cytoplasmic staining with increased cytoplasmic staining in hepatocytes around the central vein. Almost all the nuclei were strongly positive for HIF-1 α immunoreactivity. In contrast, livers of PCB 126 treated rats showed prominent cytoplasmic staining and markedly decreased nuclear staining compared to untreated control rats. These data suggest that PCB 126 treatment can alter normal HIF-1 α localization in PCB 126 treated hepatocytes *in vivo*.

PCB 126 reduces nuclear localization of HIF-1 α in HepG2 cells

Next, to extend these findings to human cells and to more deeply explore the underlying mechanisms, we were interested to assess whether PCB 126 exposure could similarly affect HIF-1 α localization in the human hepatocellular carcinoma cell line HepG2. HepG2 cells were left untreated or treated with PCB 126 and incubated in hypoxia for 24 h. Cells were lysed and fractioned to obtain cytoplasmic and nuclear extracts and then western blots were

performed to measure and compare HIF-1 α protein levels (Figure 2). We observed that untreated cells incubated in hypoxia showed increased levels of HIF-1 α in nuclear extracts compared to untreated cells in normoxia. In contrast, cells that were treated with PCB 126 prior to incubation in hypoxia showed lower levels of HIF-1 α accumulation in the nucleus. These data generally agree with the previous findings in PCB 126 treated rat livers and show that PCB 126 exposure can affect HIF-1 α cellular localization in hypoxic environments in both *in vivo* and *in vitro* settings. Ultimately, this might result in inhibited HIF-1 α -mediated responses and metabolic reprogramming due to reduced HIF-1 α localization to the nucleus.

HRE-luciferase reporter activity is perturbed by PCB 126

To further investigate the effect of PCB 126 on HIF-1 α function, HepG2 cells were transfected with a hypoxia-sensitive HRE-luciferase reporter vector and luminescence was measured in untreated and PCB 126 treated cells in different oxygen environments (Figure 3). Compared to untreated cells in normoxia, untreated cells in hypoxia showed significant induction of the HRE-luciferase reporter. However, when cells were treated with PCB 126 prior to incubation in hypoxia, the intensity of the luminescent signal was significantly reduced in hypoxia. PCB 126 treatment in normal oxygen resulted in only 1.8-fold induction of HRE-luciferase reporter activity. These results show that treatment with PCB 126 does interfere with HIF-1 α function in hypoxia.

Glucose consumption is reduced upon PCB 126 treatment

As we observed a reduction in HIF-1 α localization to the nucleus and inhibition of HIF-1 α function upon PCB 126 treatment, we wanted to analyze whether PCB 126 could affect hypoxia-induced cellular metabolic processes. HepG2 cells were treated with PCB 126 prior to incubating cells in hypoxia and glucose consumption was measured as a metabolic readout (Figure 4). As expected, glucose consumption was increased in untreated cells incubated in hypoxia compared to cells incubated in normal oxygen. Interestingly, pretreatment with PCB 126 resulted in a significant decrease in glucose consumption compared to untreated cells in hypoxia. These findings suggest that PCB 126 can significantly inhibit HIF-1 α -regulated cellular responses in low oxygen environments and thus can potentially contribute to metabolic reprogramming that could predispose to various pathologies.

Discussion

HIF-1 α is a crucial regulator of O₂ homeostasis and governs the expression of numerous target genes to adjust angiogenic, proliferative, or metabolic processes to changes in oxygen availability. The importance of HIF-1 α in counteracting cellular stresses is well established. However, given the crosstalk between the AhR and HIF-1 α via their common cofactor ARNT, few studies have assessed whether exposure to PCBs, particularly PCB 126, has an effect on HIF-1 α -regulated processes. In this study, we examined the effect of PCB 126 on HIF-1 α and consequences for cellular metabolism regulated by HIF-1 α . Here we show that exposure to PCB 126 affected HIF-1 α localization in rat liver hepatocytes and HIF-1 α -regulated metabolic processes in human liver-derived HepG2 cells. Our results show that the cellular localization of HIF-1 α within rat livers was markedly shifted towards the cytoplasm

after PCB 126 exposure compared to livers of untreated rats which showed primarily nuclear localization of HIF-1 α . Similar findings were obtained from PCB 126 treated human HepG2 cells *in vitro* which showed reduced nuclear HIF-1 α protein levels in hypoxia compared to untreated cells in hypoxia. A possible mechanism of HIF-1 α relocation might be insufficient availability of ARNT to bind to HIF-1 α . It is possible that the AhR, activated by PCB 126 exposure, dimerizes with ARNT thus sequestering it from HIF-1 α which blunts the activation of HIF-1 α -mediated downstream signaling. Unbound HIF-1 α then gets exported from the nucleus into the cytoplasm, possibly for subsequent degradation. Alternatively, it is possible that PCB 126 treatment affects proteins or other relevant factors that are involved in the transport of HIF-1 α into the nucleus which would also result in reduced nuclear localization of HIF-1 α . Importantly, reduced nuclear localization might ultimately affect the expression of HIF-1 α -regulated target genes or the activation and reprogramming of metabolic processes due to the reduced availability of HIF-1 α .

Based on these finding we next asked whether PCB 126 treatment could affect HIF-1 α function in hypoxic environments. Cells transfected with a HRE-luciferase reporter vector showed reduced induction of the reporter gene in PCB 126 treated cells in hypoxia compared to untreated cells in hypoxia. These data suggest that treatment with PCB 126 can negatively affect HIF-1 α -regulated processes and support our later findings that PCB 126 likely affects cellular metabolism by inhibiting HIF-1 α -mediated responses. Changes in the oxygen environment do not only lead to activation of HIF-1 α , but can also be associated with oxidative stress. For example, reactive oxygen species (ROS) can inhibit PHD enzymes and thus can affect the stability of HIF-1 α (Majmundar *et al.*, 2010). As a mechanism of protection, HIF-1 α changes the expression of numerous genes in order to protect the cell against oxidative damage and to ensure cell survival. For example, metabolic processes are shifted to non-oxidative forms of glucose metabolism, and oxidative phosphorylation is reduced (Taylor, 2008). Furthermore, HIF-1 α can redirect the use of glucose through the pentose phosphate pathway (PPP). The PPP is an important source of the reducing equivalent nicotinamide adenine dinucleotide phosphate (NADPH) which is used by numerous antioxidant and other enzymes. Also, glucose is metabolized to ribose-5-phosphate (R5P) which is used as a building block for RNA and DNA (Majmundar *et al.*, 2010).

Given the importance of adaptations in glucose metabolism in hypoxia and given the strong effect of PCB 126 on HIF-1 α in rat hepatocytes, we next sought to assess the effect of PCB 126 on HIF-1 α -regulated metabolic processes, more specifically glucose consumption. Glucose uptake was measured using the fluorescent glucose analog 2-NBDG. Cells incubated in hypoxia showed increased glucose uptake compared to cells in normoxia. Pretreatment with PCB 126 significantly reduced glucose uptake in hypoxia. Interestingly, glucose uptake was inhibited by approximately 20%. Similar levels of inhibition were observed when we measured HIF-1 α protein levels. It is possible that through the crosstalk with ARNT, pretreatment with PCB 126 leads to reduced activation of HIF-1 α in hypoxic conditions or to HIF-1 α relocation to the cytoplasm. In turn, due to the reduced availability of HIF-1 α , metabolic responses regulated by HIF-1 α are impaired.

Overall, our data suggest that exposure of hepatocytes to PCB 126 has significant negative effects on HIF-1 α and HIF-1 α -regulated cellular processes. We hypothesize that treatment with PCB 126 leads to reduced HIF-1 α localization to the nucleus. The reduced availability of HIF-1 α subsequently impairs the induction of HIF-1 α -regulated metabolic processes such as glucose uptake into the cell. This study establishes an important role of PCB 126 in the regulation of HIF-1 α -regulated cellular processes *in vitro* and *in vivo* and shows that metabolic processes can be negatively affected by xenobiotic exposure. In future studies we would like to assess the duration of the inhibitory effect of PCB 126 treatment on HIF-1 α . Some studies suggested that HIF-1 α has a higher affinity for ARNT compared to the AhR (Gradin *et al.*, 1996; Fleming *et al.*, 2009). If HIF-1 α dominates over the AhR when competing for ARNT, it is plausible that exposure to AhR-inducing ligands does not have a long lasting inhibitory effect on HIF-1 α . Therefore it would be worthwhile to perform temporal studies on the inhibitory effect of PCB 126 in more detail.

Conclusions

Proper expression and function of HIF-1 α plays a critical role in the regulation of adaptive responses to low oxygen environments and is crucial for cell survival. The liver contains hypoxic zones under normal conditions and is also a major site of xenobiotic metabolism. Given the broad effects and health risks of PCBs, it is important to study how exposure to these environmental chemicals might affect cellular metabolic processes in the liver. The results of this study show that PCB 126 can indeed affect HIF-1 α -regulated responses. Our data suggest that exposure to PCB 126 can significantly perturb the normal localization and function of HIF-1 α which might ultimately affect important cellular metabolic processes and thus might potentially contribute to metabolic reprogramming that could predispose to various pathologies.

Acknowledgments

This work was supported by grant #7-13-IN-12 from the American Diabetes Association (FED) and by grant P42 ES013661-5099 from the National Institutes of Health (FED). SUV received salary support from SRP Training Core P42 ES013661-5110. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abbreviations

AhR	aryl hydrocarbon receptor
ARNT	aryl hydrocarbon receptor nuclear translocator
DMSO	dimethyl sulfoxide
HIF-1α	hypoxia-inducible factor-1 α
HRE	hypoxia response element
PCB	polychlorinated biphenyl
PCB 126	3,3',4,4',5-pentachlorobiphenyl
qRT-PCR	quantitative real-time reverse transcription polymerase chain reaction

RPLP0	ribosomal protein, large, P0
2-NBDG	2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose

References

- Dery MA, Michaud MD, Richard DE. Hypoxia-inducible factor 1: regulation by hypoxic and non-hypoxic activators. *Int. J. Biochem. Cell Biol.* 2005; 37:535–540. [PubMed: 15618010]
- Fleming CR, Billiard SM, Di Giulio RT. Hypoxia inhibits induction of aryl hydrocarbon receptor activity in topminnow hepatocarcinoma cells in an ARNT-dependent manner. *Comp Biochem Physiol C Toxicol Pharmacol.* 2009; 150:383–389. [PubMed: 19539049]
- Gordan JD, Thompson CB, Simon MC. HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. *Cancer Cell.* 2007; 12:108–113. [PubMed: 17692803]
- Gradin K, McGuire J, Wenger RH, Kvietikova I, Whitelaw ML, Toftgard R, Tora L, Gassmann M, Poellinger L. Functional interference between hypoxia and dioxin signal transduction pathways: competition for recruitment of the Arnt transcription factor. *Mol. Cell. Biol.* 1996; 16:5221–5231. [PubMed: 8816435]
- Kewley RJ, Whitelaw ML, Chapman-Smith A. The mammalian basic helix-loop-helix/PAS family of transcriptional regulators. *Int. J. Biochem. Cell Biol.* 2004; 36:189–204. [PubMed: 14643885]
- Kraemer LD, Schulte PM. Prior PCB exposure suppresses hypoxia-induced up-regulation of glycolytic enzymes in *Fundulus heteroclitus*. *Comp Biochem Physiol C Toxicol Pharmacol.* 2004; 139:23–29. [PubMed: 15556062]
- Lai IK, Dhakal K, Gadupudi GS, Li M, Ludewig G, Robertson LW, Olivier AK. N-acetylcysteine (NAC) diminishes the severity of PCB 126-induced fatty liver in male rodents. *Toxicology.* 2012; 302:25–33. [PubMed: 22824115]
- Macheda ML, Rogers S, Best JD. Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J. Cell. Physiol.* 2005; 202:654–662. [PubMed: 15389572]
- Majmundar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. *Mol. Cell.* 2010; 40:294–309. [PubMed: 20965423]
- Maxwell PH, Dachs GU, Gleadle JM, Nicholls LG, Harris AL, Stratford IJ, Hankinson O, Pugh CW, Ratcliffe PJ. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proc. Natl. Acad. Sci. U. S. A.* 1997; 94:8104–8109. [PubMed: 9223322]
- Mole DR, Blancher C, Copley RR, Pollard PJ, Gleadle JM, Ragoussis J, Ratcliffe PJ. Genome-wide association of hypoxia-inducible factor (HIF)-1 α and HIF-2 α DNA binding with expression profiling of hypoxia-inducible transcripts. *J. Biol. Chem.* 2009; 284:16767–16775. [PubMed: 19386601]
- Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, Kaelin WG. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat Cell Biol.* 2000; 2:423–427. [PubMed: 10878807]
- Oinonen T, Lindros KO. Zonation of hepatic cytochrome P-450 expression and regulation. *Biochem. J.* 1998; 329(Pt 1):17–35. [PubMed: 9405271]
- Rankin EB, Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. *Cell Death Differ.* 2008; 15:678–685. [PubMed: 18259193]
- Salnikow K, An WG, Melillo G, Blagosklonny MV, Costa M. Nickel-induced transformation shifts the balance between HIF-1 and p53 transcription factors. *Carcinogenesis.* 1999; 20:1819–1823. [PubMed: 10469629]
- Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 2012; 9:671–675. [PubMed: 22930834]
- Semenza GL. Regulation of mammalian O₂ homeostasis by hypoxia-inducible factor 1. *Annu. Rev. Cell Dev. Biol.* 1999; 15:551–578. [PubMed: 10611972]

- Taylor CT. Mitochondria and cellular oxygen sensing in the HIF pathway. *Biochem. J.* 2008; 409:19–26. [PubMed: 18062771]
- Vorrink SU, Severson PL, Kulak MV, Futscher BW, Domann FE. Hypoxia perturbs aryl hydrocarbon receptor signaling and CYP1A1 expression induced by PCB 126 in human skin and liver-derived cell lines. *Toxicol. Appl. Pharmacol.* 2013
- Wood IS, Stezhka T, Trayhurn P. Modulation of adipokine production, glucose uptake and lactate release in human adipocytes by small changes in oxygen tension. *Pflugers Arch.* 2011; 462:469–477. [PubMed: 21698390]

A **Control 20x** **Control 40x****B** **PCB 126 20x** **PCB 126 40x****Figure 1. Parenteral exposure to PCB 126 affects HIF-1α localization in rat livers**

Formalin-fixed, paraffin-embedded liver sections of male Sprague-Dawley rats treated with PCB 126 as described in Materials and Methods were stained for HIF-1α. Arrows indicate nuclei with strong HIF-1α staining. Scale bars = 50 μm in 20x and 20 μm in 40x micrographs.

Figure 2. Nuclear localization of HIF-1α is reduced after PCB 126 treatment

HepG2 cells were treated with 3 μM PCB 126 for 4 h prior to incubation in hypoxia for 24 h. Cells were lysed and fractionated into nuclear and cytoplasmic components. Twenty micrograms of cytoplasmic or nuclear proteins were western blotted for HIF-1α (top panel). Betatubulin (middle panel) was used as loading control for cytoplasmic extracts and histone H3 (bottom panel) was used as loading control for nuclear extracts. Relative signal intensities of HIF-1α compared to untreated cells in hypoxia were calculated and are depicted as numbers below the blot. CE = cytoplasmic extract; NE = nuclear extract.

Figure 3. PCB 126 treatment inhibits HRE-luciferase reporter activity in hypoxia

HepG2 cells were transfected with a HRE-luciferase (firefly) reporter vector and Renilla luciferase vector. Transfected cells were treated with 3 μ M PCB 126 for 4 h and then subjected to hypoxia for 10 h. The luminescent signal was measured and is depicted relative to untreated cells in normoxia. Values significantly ($p < 0.05$) greater than untreated controls in normoxia are indicated by an asterisk (*). Values significantly ($p < 0.05$) different between the PCB 126 treated samples in hypoxia and hypoxia alone are indicated by a number sign (#). Error bars = SEM. n = 4.

Figure 4. PCB 126 treatment decreases glucose consumption in hypoxia

HepG2 cells were treated with 3 μM PCB 126 for 4 h prior to subjecting cells to hypoxia for 24 h. 2-NBDG fluorescence was recorded using flow cytometry and is depicted relative to untreated cells in normoxia. * = $p < 0.05$ vs. control in normoxia. # = $p < 0.05$ hypoxia plus PCB 126 vs. hypoxia alone. Error bars = SEM. n = 3.